1637

PATENT
Customer No. 22,852
Attorney Docket No. 3495.0010-24

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)
Marc ALIZON et al.	) Group Art Unit: 1637
Application No.: 08/475,822	) Examiner: Jeffrey N. FREDMAN
Filed: June 7, 1995	) Confirmation No.: 4214

For: CLONED DNA SEQUENCES RELATED TO THE GENOMIC RNA OF LYMPHADENOPATHY-ASSOCIATED VIRUS (LAV) AND PROTEINS ENCODED BY SAID LAV GENOMIC RNA

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

TRANSMITTAL LETTER

Applicants enclose the following papers. Kindly associate them with the above-identified application.

1. Petition to Suspend Action under 37 C.F.R. § 1.103 (4 pages with Exhs. 1-5).

2. $200 Check for fee required by 1.17(g).

Please grant any extensions of time required to enter this response and charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: November 28, 2005

By: _____

Salvatore J. Arrigo, Reg. No. 46,063
Tel. 202-408-4160 Fax.: 202-408-4400
E-mail: arrigos@finnegan.com

PATENT
Customer No. 22,852
Attorney Docket No. 3495.0010-24

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Marc ALIZON et al.	)	Group Art Unit: 1637
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For: CLONED DNA SEQUENCES RELATED TO THE GENOMIC RNA OF LYMPHADENOPATHY-ASSOCIATED VIRUS (LAV) AND PROTEINS ENCODED BY SAID LAV GENOMIC RNA

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

PETITION TO SUSPEND ACTION UNDER 37 C.F.R. § 1.103

Applicants respectfully request suspension of action in this application under 37 C.F.R. § 1.103 for a period of six months. This suspension is necessary to allow applicants time to determine the correct assignee(s) of the application so that a Terminal Disclaimer signed by the correct assignee(s) can be filed to overcome an outstanding rejection under the judicially created doctrine of obviousness-type double patenting in this application. Applicants' petition should be granted in view of the following facts:

1. In an Office Action dated April 25, 2005, the Office rejected claims 51 and 52 in this application under the judicially created doctrine of obviousness-type double patenting over claims 1-6 of U.S. Patent No. 6,627,395. (Exhibit 1 at 9.)

2. Applicants filed a Reply on October 25, 2005. (Exhibit 2.)

3. Thus, in compliance with 37 C.F.R. § 1.103(a), no reply to an Office Action is currently required.

4. The $200.00 fee required by 1.17(g) is enclosed.

5. In the Reply filed October 25, 2005, applicants agreed to file a Terminal Disclaimer once applicants have determined the correct inventors and ownership of this application. (Exhibit 2 at 3.)

6. The instant application is a division of application Serial No. 08/158,652, filed February 22, 1988 (pending), which is a division of application Serial No. 06/771,248, filed August 30, 1985 (now abandoned). This application is also a continuation-in-part of application Serial No. 08/999,410, filed December 31, 1992 (pending), which is a continuation of application Serial No. 07/499,210, filed March 19, 1990 (now abandoned), which is a continuation of application Serial No. 06/771,230, filed August 30, 1985 (now abandoned), which is a continuation-in-part of application Serial No. 06/706,562, filed February 28, 1985 (now abandoned), which is a continuation-in-part of application Serial No. 06/558,109, filed December 5, 1983 (now abandoned). (Exhibit 3 at 2.)

7. U.S. Patent No. 6,627,395 is a continuation of application Ser. No. 08/019,297, filed Feb. 18, 1993, which is a division of application Ser. No. 07/876,297, filed Apr. 30, 1992, now abandoned, which is a continuation application of Ser. No. 07/117,937, filed Nov. 5, 1987, now U.S. Pat. No. 5,135,864, which is a continuation application of Ser. No. 06/785,638, filed Oct. 8, 1985, now U.S. Pat.

No. 4,708,818, which is a continuation application of Ser. No. 06/558,109, filed Dec. 5, 1983, now abandoned. (Exhibit 4 at face page.)

8. U.S. Patent No. 6,627,395 is currently assigned to Institut Pasteur and The United States of America as represented by the Secretary of the Department of Health and Human Services. (Exhibit 4 at face page.)

9. In contrast, the instant application is currently assigned to Institut Pasteur and Centre National de la Recherche Scientifique. (Exhibit 5.)

10. In order to file a Terminal Disclaimer in the instant application, the instant application and U.S. Patent No. 6,627,395 must be commonly owned. (*See* 37 C.F.R. § 1.321.)

11. Filing a Terminal Disclaimer will fix the expiration date of a patent issuing from the instant application as the same expiration date of U.S. Patent No. 6,627,395.

12. Thus, a suspension of action will not extend the term of a patent issuing from the instant application.

In view of the above facts, Institut Pasteur must determine the correct assignees of the claims of the instant application and the claims of U.S. Patent No. 6,627,395 and have the appropriate legal documents executed in order to file the required Terminal Disclaimer in the instant application. The difficulty in making this determination is complicated by the fact that the earliest claimed U.S. priority date in both applications is over 20 years ago. Nonetheless, Institut Pasteur is diligently trying to resolve this issue with the other assignees. Applicants believe that the above facts establish good and sufficient cause for a suspension of action in this application under 37 C.F.R. § 1.103 to allow time for Institut Pasteur to determine the correct assignees. Accordingly,

applicants respectfully request a suspension of action for a period of six months in this application.

Please grant any extensions of time required to enter this response and charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: November 28, 2005

By: _____

Salvatore J. Arrigo
Registration No. 46,063
Telephone: 202-408-4160
Facsimile: 202-408-4400
E-mail: arrigos@finnegan.com

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/475,822	06/07/1995	MARC ALIZON	3495.0010-24	4214

EXAMINER	
FREDMAN, JEFFREY NORMAN	

ART UNIT	PAPER NUMBER
1637	

22852 7590 04/25/2005

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

DATE MAILED: 04/25/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Docketed 4/27/05 Attorney KJM/SJB
Case 3495.0010-24CON
Due Date 7/25/05 w/ext
Action Response
By

✓ pm

Office Action Summary	Application No. 08/475,822	Applicant(s) ALIZON ET AL.	
	Examiner Jeffrey Fredman	Art Unit 1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 March 2005* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *47,51 and 52* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *47,51 and 52* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 3/9/05
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Continued Examination Under 37 CFR 1.129(a)

1. This application is subject to the provisions of Public Law 103-465, effective June 8, 1995. Accordingly, since this application has been pending for at least two years as of June 8, 1995, taking into account any reference to an earlier filed application under 35 U.S.C. 120, 121 or 365(c), applicant, under 37 CFR 1.129(a), is entitled to have a first submission entered and considered on the merits if, prior to abandonment, the submission and the fee set forth in 37 CFR 1.17(r) are filed prior to the filing of an appeal brief under 37 CFR 1.192. Upon the timely filing of a first submission and the appropriate fee under 37 CFR 1.17(r), the finality of the previous Office action is withdrawn. In view of 35 U.S.C. 132, no amendment considered as a result of payment of the fee set forth in 37 CFR 1.17(r) may introduce new matter into the disclosure of the application.

Claim Rejections - 35 USC § 112 – Written Description

2. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claim 47, 51 and 52 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

In analysis of the claims for compliance with the written description requirement of 35 U.S.C. 112, first paragraph, the written description guidelines note regarding genus/species situations that "Satisfactory disclosure of a ``representative number'' depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed." (See: Federal Register: December 21, 1999 (Volume 64, Number 244), revised guidelines for written description.)

Claims 47, 51 and 52 are generic claims which are based upon a single species. That is, Applicant identified a single HIV-1 sequence. However, the claims encompass a genus of any HIV-1 nucleic acid anywhere, which genus comprises each of the hundreds of millions of different variants which exist around the world. These HIV-1 variants are not disclosed in the specification resulting in a genus which includes variants for which no written description is provided in the specification. This large genus is represented in the specification by only a single HIV-1 sequence. Thus, applicant has express possession of only a single HIV-1 sequence in a genus which comprises hundreds of millions of different possibilities.

The claim indicates no common element or attributes of the sequences that is required. No structural domains such as specific amino acid sequences, or functional domains or any common attribute whatsoever is required. Simply a virus which shares the same name as that identified by Applicant (and regarding which Applicant lost an interference to Chang et al regarding priority). Claims 47, 51 and 52 have no structural

limitations or requirements which provide guidance on the identification of sequences which even distinguish, in a structural way, HIV-1 from any other similar lentivirus such as SIV. Claims 47, 51 and 52 provide no written description of alleles, of insertions, of deletions or of any other variation in the HIV-1 sequence.

It is noted in the recently decided case The Regents of the University of California v. Eli Lilly and Co. 43 USPQ2d 1398 (Fed. Cir. 1997) decision by the CAFC that

> "A definition by function, as we have previously indicated, does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is. See Fiers, 984 F.2d at 1169- 71, 25 USPQ2d at 1605- 06 (discussing Amgen). It is only a definition of a useful result rather than a definition of what achieves that result. Many such genes may achieve that result. The description requirement of the patent statute requires a description of an invention, not an indication of a result that one might achieve if one made that invention. See In re Wilder, 736 F.2d 1516, 1521, 222 USPQ 369, 372- 73 (Fed. Cir. 1984) (affirming rejection because the specification does "little more than outlin[e] goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate."). Accordingly, naming a type of material generally known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material. "

In the current situation, the definition of the sequence a an HIV-1 sequence, without any specific structure given in claims 47, 51 and 52, is precisely the situation of naming a type of material which is generally known to likely exist, but except for the single specific example of a particular HIV-1 sequence provided in the specification, is in the absence of knowledge of the material composition and fails to provide descriptive support for the generic claim.

It is noted that in Fiers v. Sugano (25 USPQ2d, 1601), the Fed. Cir. concluded that

> "...if inventor is unable to envision detailed chemical structure of DNA sequence coding for specific protein, as well as method of obtaining it, then conception is not achieved until reduction to practice has occurred, that is, until after gene has been isolated...conception of any chemical substance, requires definition of that substance other than by its functional utility."

The current situation is a definition of the compound solely but its functional utility, as an HIV-1 sequence, without any definition of the particular sequences claimed. Claims 47, 51 and 52 envision a scope which encompasses any HIV-1 sequence but the specification does not provided the detailed chemical structure of any DNA other than the single HIV-1 sequence described.

In the instant application, a single specific sequence is described. Also, in Vas-Cath Inc. v. Mahurkar (19 USPQ2d 1111, CAFC 1991), it was concluded that:

> "...applicant must also convey, with reasonable clarity to those skilled in art, that applicant, as of filing date sought, was in possession of invention, with invention being, for purposes of "written description" inquiry, whatever is presently claimed."

In the application at the time of filing, there is no record or description which would demonstrate conception of any nucleic acids other than the single sequence expressly disclosed. Therefore, claim 47 fails to meet the written description requirement by encompassing sequences which are not described in the specification.

Claim Rejections - 35 USC § 112 – Enablement

4. Claims 47, 51 and 52 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for detection of the specific HIV-1 sequence disclosed in the specification, does not reasonably provide enablement for detection of

HIV-1 variants which are not disclosed in the specification. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention

The claims are drawn to a methods of detecting the presence of HIV-1 in a subject comprising the steps of detecting HIV-1 nucleic acids present in the supernatant of a biological sample. The invention is in a class of invention which the CAFC has characterized as "the unpredictable arts such as chemistry and biology." Mycogen Plant Sci., Inc. v. Monsanto Co., 243 F.3d 1316, 1330 (Fed. Cir. 2001).

The breadth of the claims

The claims encompass diagnosis of any HIV-1 virus, whether there is significant shared sequence or not. The claim includes no structural elements whatsoever regarding the Human Immunodeficiency virus. No specific polymorphisms in HIV-1 or sequence alterations are identified. The claims encompass any insertion, any deletion, any substitution or any alteration whatsoever relative to the HIV-1 sequence disclosed

in the specification. No specific sequences are recited for the HIV-1 sequences are provided so these claim terms broadly encompass any sequence which can be so named.

Quantity of Experimenation

The quantity of experimentation in this area is extremely large since determination of the diagnostic efficacy of any particular HIV-1 sequence with relation to the presence of the virus would require identification of a disease cohort, since different individuals are infected with different subtypes of the HIV-1 virus and different probes would function to detect different subtypes or other variants. In the case of HIV-1 polymorphisms, deletions, insertions and other sequence alterations, analysis of the entire cohort for the alteration would be required and performance of this method on a large enough sample to be statistically significant. This would require significant inventive effort, with each of the many intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps.

The unpredictability of the art and the state of the prior art

The prior art shows that a probe which detects one strain of HIV-1 may fail to detect other HIV-1 strains. Candotti et al (AIDS (1991) 5(8):1003-7) notes "Moreover, the DNA amplified from two other isolates did not hybridize with the corresponding probe despite efficient PCR. Base substitutions were detected in the regions of proviral genomes involved in oligonucleotide annealing and were assumed to be responsible for the **failure** of both amplification and probing. Our data confirm that the genetic variability of HIV-1 may reduce the efficiency of PCR as a diagnostic procedure,

especially in the case of African isolates (emphasis added)." So even 8 years after the filing date of the specification from which priority is claimed, there was significant variability in the detection of HIV-1 using HIV-1 nucleic acids. This unpredictability is heightened with regard to the current application which was written in a time when oligonucleotide synthesis was uncertain and difficult, the PCR method used by Candotti had not yet been invented, and probe synthesis, selection and detection methods were significantly more primitive than they were in 1991, much less now in 2004.

Working Examples

The specification has one working example of an HIV-1 sequence. There are no other working examples.

Guidance in the Specification.

The specification, while providing a general review of methods to diagnose HIV-1 does not provide teachings sufficient to overcome doubts raised in the art with regards to the unpredictability of probes to function and with regard to the absence of any sequence for any HIV-1 other than the single sequence disclosed. It would essentially be a trial and error process to make and use the many possible diverse species of HIV-1 encompassed by the claims in order to diagnose disease.

Level of Skill in the Art

The level of skill in the art is deemed to be high.

Conclusion

In the instant case, as discussed above, the level of unpredictability in the art is high as shown by the cited prior art, the specification provides one with little description

or guidance that leads one to a reliable method of diagnosis of HIV-1. One of skill in the art cannot readily anticipate the effect of a change within the subject matter to which the claimed invention pertains. Further the specification does not provide guidance to overcome art recognized problems in diagnosis required to actually use the diagnostic methods as broadly claimed for all HIV-1 nucleic acids whatever their sequence. Thus given the broad claims in an art whose nature is identified as unpredictable, the unpredictability of that art, the large quantity of research required to define these unpredictable variables, the lack of guidance provided in the specification, the small number of working examples and the negative teachings in the prior art balanced only against the high skill level in the art, it is the position of the examiner that it would require undue experimentation for one of skill in the art to perform the method of the claim as broadly written.

Double Patenting

5. Claims 51 and 52 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-6 of U.S. Patent No. 6,627,395 Although the conflicting claims are not identical, they are not patentably distinct from each other because the issued claims represent a species which anticipates the current generic claims.

Claims 1-6 of U.S. Patent No. 6,627,395 teach a method for preparing and detecting HIV-1 RNA from a lysate of an HIV-1 virus, said method comprising: (a) providing a biological sample that comprises human CD4+ lymphocytes infected with HIV-1 virus; (b) separating said virus from said human CD4+ lymphocytes; (c) centrifuging said separated virus to form a fraction comprising concentrated virus; (d)

isolating said fraction comprising concentrated virus; (e) lysing said virus; (f) precipitating the RNA of said virus; and (g) detecting said viral RNA.

2. The method of claim 1, wherein said method comprises banding said virus on a sucrose gradient or a metrizamide gradient.

3. The method of claim 1, wherein said method comprises pelleting said virus.

4. The method of claim 3, wherein said method comprises precipitating said virus with polyethylene glycol.

5. The method of claim 1, wherein the virus is lysed with SDS.

6. The method of claim 1, wherein said nucleic acid is precipitated with trichloroacetic acid.

6. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Response to Arguments

7. Applicant's arguments filed March 9, 2005 have been fully considered but they are not persuasive.

Applicant simply argues that the claims are allowable. The rejections above contravert that position.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jeffrey Fredman whose telephone number is (571)272-0742. The examiner can normally be reached on 6:30-3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571)272-0782. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Jeffrey Fredman
Primary Examiner
Art Unit 1637
7/12/05

IDS Form PTO/SB/08: Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	08/475,822
Filing Date	June 7, 1995
First Named Inventor	Marc ALIZON et al.
Art Unit	1637
Examiner Name	Jeffrey N. FREDMAN
Attorney Docket Number	3495.0010-24

U.S. PATENTS AND PUBLISHED U.S. PATENT APPLICATIONS

Examiner Initials	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
JN		US 6,627,395 B1-	09/30/2003	Montagnier et al.	

Note: Copies of the U.S. Patent Documents are not Required in IDS filed after October 21, 2004

FOREIGN PATENT DOCUMENTS

Examiner Initials	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[6]

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation[6]
JN		Paper No. 300 in Interference No. 102,822; APJs Metz, Pate, and Martin; 03/09/2001; pp. 1-6.	
		Paper No. 271 in Interference No. 102,822; APJs Metz, Pate, and Martin; 04/21/1999; pp. 1-3.	
		Paper No. 282 in Interference No. 102,822; APJs Metz, Pate, and Martin; 07/30/1999; pp. 1-2.	
		Paper No. 289 in Interference No. 102,822; APJs Metz, Pate, and Martin; 10/19/1999; pp. 1-3.	
		Copies of claims 30, 31, 57-61, 109-115, and 132 in Interference No. 102,822.	

Examiner Signature		Date Considered	4/10-5

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

PATENT
Customer No. 22,852
Attorney Docket No. 3495.0010-24

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Marc ALIZON et al.	Group Art Unit: 1637
Application Ser. No.: 08/475,822	Examiner: Jeffrey N. FREDMAN
Filed: June 7, 1995	Confirmation No.: 4214

For: CLONED DNA SEQUENCES RELATED TO THE GENOMIC RNA OF LYMPHADENOPATHY-ASSOCIATED VIRUS (LAV) AND PROTEINS ENCODED BY SAID LAV GENOMIC RNA

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RESPONSE

In response to the Office Action dated April 25, 2005, the period for response to which has been extended by filing a Petition for Extension of Time and fee concurrently herewith, applicants submit the following remarks.

REMARKS

Reconsideration of this application is respectfully requested.

Claims 47, 51, and 52 are pending in this application.

Claims 47, 51, and 52 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to the skilled artisan that the inventors had possession of the claimed invention at the time the application was filed. The Office alleges that the claims encompass a genus of HIV-1 variants, which are not disclosed in the specification.

Applicants traverse the rejection. Claims 47, 51, and 52 are directed to ***generic methods*** for preparing and detecting the presence of HIV-1 RNA. These methods do not require knowledge of the sequence of the HIV-1 virus that is prepared and detected. Thus, applicants need not provide the sequence of all HIV-1 viruses to describe a generic method that will work with all of these viruses. Moreover, the Office has set forth no reasons to doubt that the claimed methods will work regardless of the sequence of the HIV-1 virus that is prepared and detected. Accordingly, applicants respectfully request withdrawal of the rejection.

Claims 47, 51, and 52 were rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for detection of the specific HIV-1 sequences disclosed in the specification, allegedly does not reasonably provide enablement for detection of HIV-1 variants that are not disclosed in the specification.

Applicants traverse the rejection. First, claim 51 does not require "detecting" HIV-1. Thus, the basis for the Office's rejection of claim 51 is in error, and applicants respectfully request withdrawal of the rejection.

Second, as long as applicants' specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. § 112, first paragraph, is satisfied. *See In re Fisher*, 166 U.S.P.Q. 18, 24 (C.C.P.A. 1970). Applicants' specification fulfills this requirement. The methods of claims 47, 50 and 51 do not require knowledge of the sequence of the HIV-1 virus that is prepared and detected. The Office has set forth no reasons to doubt that the claimed methods will work regardless of the sequence of the HIV-1 virus that is prepared and detected. Accordingly, applicants respectfully request withdrawal of the rejection.

Claims 51 and 52 were rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-6 of U.S. Patent No. 6,627,395. Solely to expedite prosecution of this application and not in acquiescence to this rejection, applicants agree to file a Terminal Disclaimer once applicants have determined the correct inventors and ownership of this application.

Applicants respectfully submit that this application is in condition for allowance. In the event that the Examiner disagrees, he is invited to call the undersigned to discuss any outstanding issues remaining in this application in order to expedite prosecution.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: October 25, 2005

By: _____

Salvatore J. Arrigo
Reg. No. 46,063
Telephone: 202-408-4160
Facsimile: 202-408-4400
E-mail: arrigos@finnegan.com

PATENT
Customer No. 22,852
Attorney Docket No. 3495.0010-24

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Marc ALIZON et al.	Group Art Unit: 1634
Application Ser. No.: 08/475,822	Examiner: Jeffrey Norman Fredman
Filed: June 7, 1995	Confirmation No.: 4214

For: CLONED DNA SEQUENCES RELATED TO THE GENOMIC RNA OF LYMPHADENOPATHY-ASSOCIATED VIRUS (LAV) AND PROTEINS ENCODED BY SAID LAV GENOMIC RNA

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

AMENDMENT AND RESPONSE

In response to the Office Action dated February 13, 2004, applicants submit the following amendments and remarks. Please amend this application as follows.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims in this paper.

Remarks begin on page 5 of this paper.

AMENDMENTS TO THE SPECIFICATION:

Please replace the first paragraph of the specification with the following amended paragraph:

This application is a division of application Serial No. 07/158,652, filed February 22, 1988 (pending), which is a division of Serial No. 06/771,248, filed August 30, 1985 (now abandoned). This application is also a continuation-in-part of application serial no. 07/999,410, filed December 31, 1992 (pending), which is a continuation of application Serial No. 07/499,210 filed March 19, 1990 (now abandoned), which is a continuation of application Serial No. 06/771,230, filed August 30, 1985 (now abandoned), which is a continuation-in-part of application Serial No. 06/706,562, filed February 28, 1985 (now abandoned), which is a continuation-in-part of application Serial No. 06/558,109, filed December 5, 1983 (now abandoned).

AMENDMENTS TO THE CLAIMS:

The following listing of claims replaces all prior versions of the claims.

LISTING OF CLAIMS:

1-34. (canceled)

35. (currently amended) An *in vitro* diagnostic method for detecting the presence ~~or absence~~ of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) in a biological sample comprising:

(a) providing a cell-free supernatant of a biological fluid comprising cells infected with HIV-1, and

~~(a)~~ (b) contacting said ~~biological sample~~ supernatant with one or more nucleic acid probes comprising

(i) a nucleic acid of ORF-1 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MEQAPEDQGPQREPHNEWTLELLEELKNEAVRHFPRIWLHGLGQHIYETYGDT WAGVEAIIRILQQLLFIHFRIGCRHSRIGVTQQRRARNGASRS,

(ii) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL, and

(iii) a nucleic acid of ORF-R of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAAT NAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDI

LDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLH PVSLHGMDDPEREVLEWRFDSRLAFHHVARELHPEYFKNC; and

~~(b)~~ (c) detecting the formation of hybrids between said one or more nucleic acid probes and HIV-1 nucleic acid present in said ~~biological sample~~ supernatant.

36. (previously presented) The method according to claim 35, wherein said probe is labeled with a label selected from the group consisting of a radioactive label, an enzymatic label, and a fluorescent label.

37. (currently amended) An *in vitro* diagnostic method for detecting the presence ~~or absence~~ of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) in a biological sample comprising:

(a) providing a cell-free supernatant of a biological fluid comprising cells infected with HIV-1, and

~~(a)~~ (b) contacting said ~~biological sample~~ supernatant with one or more nucleic acid probes comprising

(i) a nucleic acid of ORF-1 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MEQAPEDQGPQREPHNEWTLELLEELKNEAVRHFPRIWLHGLGQHIYETYGDT WAGVEAIIRILQQLLFIHFRIGCRHSRIGVTQQRRARNGASRS and

(ii) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL; and

~~(b)~~ (c) detecting the formation of hybrids between said one or more nucleic acid probes and HIV-1 nucleic acid present in said ~~biological sample~~ supernatant.

38. (previously presented) The method according to claim 37, wherein said probe is labeled with a label selected from the group consisting of a radioactive label, an enzymatic label, and a fluorescent label.

39. (currently amended) An *in vitro* diagnostic method for detecting the presence ~~or absence~~ of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) in a biological sample comprising:

(a) providing a cell-free supernatant of a biological fluid comprising cells infected with HIV-1, and

~~(a)~~ (b) contacting said ~~biological sample~~ supernatant with one or more nucleic acid probes comprising

(i) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL and

(ii) a nucleic acid of ORF-R of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAAT NAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDI LDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLH PVSLHGMDDPEREVLEWRFDSRLAFHHVARELHPEYFKNC; and

~~(b)~~ (c) detecting the formation of hybrids between said one or more nucleic acid probes and HIV-1 nucleic acid present in said ~~biological sample~~ supernatant.

40. (previously presented) The method according to claim 39, wherein said probe is labeled with a label selected from the group consisting of a radioactive label, an enzymatic label, and a fluorescent label.

41. (previously presented) An *in vitro* diagnostic kit for detecting the presence or absence of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) in a biological sample comprising:

(a) a composition comprising one or more nucleic acid probes comprising

(i) a nucleic acid of ORF-1 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MEQAPEDQGPQREPHNEWTLELLEELKNEAVRHFPRIWLHGLGQHIYETYGDT WAGVEAIIRILQQLLFIHFRIGCRHSRIGVTQQRRARNGASRS,

(ii) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL, and

(iii) a nucleic acid of ORF-R of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAAT NAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDI LDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLH PVSLHGMDDPEREVLEWRFDSRLAFHHVARELHPEYFKNC;

(b) reagents for detecting the hybrids; and

(c) a biological reference sample lacking nucleic acid recognized by said nucleic acid probe composition.

42. (previously presented) The kit according to claim 41, wherein said probe is labeled with a label selected from the group consisting of a radioactive label, an enzymatic label, and a fluorescent label.

43. (previously presented) An *in vitro* diagnostic kit for detecting the presence or absence of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) in a biological sample comprising:

(a) a composition comprising one or more nucleic acid probes comprising

(i) a nucleic acid of ORF-1 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MEQAPEDQGPQREPHNEWTLELLEELKNEAVRHFPRIWLHGLGQHIYETYGDT WAGVEAIIRILQQLLFIHFRIGCRHSRIGVTQQRRARNGASRS and

(ii) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL;

(b) reagents for detecting the hybrids; and

(c) a biological reference sample lacking nucleic acid recognized by said nucleic acid probe composition.

44. (previously presented) The kit according to claim 43, wherein said probe is labeled with a label selected from the group consisting of a radioactive label, an enzymatic label, and a fluorescent label.

45. (previously presented) An *in vitro* diagnostic kit for detecting the presence or absence of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) in a biological sample comprising:

(a) a composition comprising one or more nucleic acid probes comprising

(i) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL and

(ii) a nucleic acid of ORF-R of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAAT NAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDI LDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLH PVSLHGMDDPEREVLEWRFDSRLAFHHVARELHPEYFKNC;

(b) reagents for detecting the hybrids; and

(c) a biological reference sample lacking nucleic acid recognized by said nucleic acid probe composition.

46. (previously presented) The kit according to claim 45, wherein said probe is labeled with a label selected from the group consisting of a radioactive label, an enzymatic label, and a fluorescent label.

47. (new) An *in vitro* diagnostic method for detecting the presence of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) in a biological sample comprising:

(a) providing a cell-free supernatant of a biological fluid comprising cells infected with HIV-1, and

(b) detecting HIV-1 nucleic acid present in said supernatant.

48. (new) The *in vitro* diagnostic method of claim 47, wherein the presence of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) is detected by

(a) contacting said supernatant with one or more nucleic acid probes comprising:

(i) a nucleic acid of ORF-1 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MEQAPEDQGPQREPHNEWTLELLEELKNEAVRHFPRIWLHGLGQHIYETYGDT WAGVEAIIRILQQLLFIHFRIGCRHSRIGVTQQRRARNGASRS,

(ii) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL, and

(iii) a nucleic acid of ORF-R of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAAT NAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDI

LDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLH PVSLHGMDDPEREVLEWRFDSRLAFHHVARELHPEYFKNC; and

(b) detecting the formation of hybrids between said one or more nucleic acid probes and HIV-1 nucleic acid present in said supernatant.

~~49. (new) The *in vitro* diagnostic method of claim 47~~, wherein the presence of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) is detected by

(a) contacting said supernatant with one or more nucleic acid probes comprising:

(i) a nucleic acid of ORF-1 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MEQAPEDQGPQREPHNEWTLELLEELKNEAVRHFPRIWLHGLGQHIYETYGDT WAGVEAIIRILQQLLFIHFRIGCRHSRIGVTQQRRARNGASRS and

(ii) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL; and

(b) detecting the formation of hybrids between said one or more nucleic acid probes and HIV-1 nucleic acid present in said supernatant.

50. (new) The *in vitro* diagnostic method of claim 47, wherein the presence of nucleic acid of a Human Immunodeficiency Virus Type 1 (HIV-1) is detected by

(a) contacting said supernatant with one or more nucleic acid probes comprising:

(i) a nucleic acid of ORF-4 of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MQPIQIAIAALVVAIIIAIVVWSIVIIEYRKILRQRKIDRLIDRLIERAEDSGNESEGEIS ALVEMGVEMGHHAPWDIDDL and

(ii) a nucleic acid of ORF-R of Human Immunodeficiency Virus Type 1 (HIV-1) encoding the amino acid sequence:

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAAT NAACAWLEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDI LDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPVEPDKVEEANKGENTSLLH PVSLHGMDDPEREVLEWRFDSRLAFHHVARELHPEYFKNC; and

(b) detecting the formation of hybrids between said one or more nucleic acid probes and HIV-1 nucleic acid present in said supernatant.

REMARKS

Reconsideration of this application is respectfully requested.

Claims 35, 37, and 39 have been amended. Claims 47-50 are new and are derived from claims 35-46. No new matter enters by amendment.

~~Claims 35, 37, 39, 41, 43, and 45 were rejected under 35 U.S.C. § 102(e)~~ as allegedly being anticipated by Chang (U.S. Patent No. 6,001,977) and claims 35-46 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Chang et al. in view of White et al. (U.S. Patent No. 4,677,054). The Examiner's position rests on the allegation that applicants' LAV strain and Chang's HTLV-III strain came from the same person and represent different isolates of the same virus. Based on this allegation, the Examiner concludes that differences between Chang's sequence and applicants' sequence are due to "sequencing errors."

Applicants traverse the rejection. There is no evidence of record to support the Examiner's conclusion. Rather, the evidence of record supports the opposite conclusion. First, the sequences of applicants' LAV clone and Chang's HTLV-III clones are different. (*See* U.S. Patent No. 6,001,977.) Second, the extent of differences is higher than would be expected for sequencing errors. (*See* Applicants' August 1, 2003, Amendment at 3-5.) Third, Ratner et al. indicates that differences in the sequence of clones of HIV-1 need not be due to sequencing errors. (Ratner et al. at 59.)

The Examiner's conclusion that Ratner et al. does not support applicants' position is in error. Ratner et al. found a substantial number (79) of differences between two different clones of HTLV-III (HXB2 and BH10). Ratner et al. indicates that these differences are unlikely "to represent cloning artifacts or sequencing errors since 1)

these alterations were confirmed by DNA sequences from both strands of both clones, and 2) 82% of these changes are present in other previously sequenced HTLVIII/LAV clones." (Ratner et al. at 59, ¶ 4.) Since Ratner et al. shows that two different clones of the same strain (HTLV-III) can have substantial differences in sequence, there is no reason to believe that ~~the differences between applicants' LAV~~ clone and Chang's HTLV-III clone must be due to sequencing errors.

Similar to the sequence of BH10 and BH5 in the '977 patent (*see* Table 3 in Applicants' September 3, 2002, Amendment and Response), Ratner's sequence contains a frameshift in the Vpr orf due to an additional "t" at position ~5350. (Ratner et al. at 61, Figure 1.) Thus, three different clones of HTLV-III contain the same difference from applicants' strain. Consequently, Ratner et al. supports that Chang's BH10 clone does not encode applicants' Vpr. Accordingly, applicants' claimed nucleic acids expressing Vpr cannot be anticipated by Chang.

Furthermore, Muesing et al. (Exhibit 1) independently isolated cDNA and proviral clones of HTLV-III from the H9/HTLVIII cell line. (Muesing et al. at 450, col. 2.) The sequence of HTLV-III presented in Figure 3 shows that Muesing's clones, similar to Chang's clones, contain a frameshift in the Vpr orf due to an additional "t" at position ~5350. (*Id.* at 453, Figure 3.) Thus, Muesing et al. provides evidence that the virus or viruses in the H9/HTLVIII cell line, from which Chang's clones were derived, do not encode applicants' Vpr. Accordingly, applicants' claimed nucleic acids expressing Vpr cannot be anticipated by Chang.

Applicants' LAV strain and Chang's HTLV-III strain may have come from the same person and may represent different isolates of the same virus. However, the

issue in this case is whether the sequence of applicants' **clones** and Chang's **clones** are the same. The Examiner's conclusion ignores the potential for the introduction of nucleotide sequence changes during virus replication *in vivo* and *in vitro* during preparation of the clones (*see, e.g.*, Goodenow et al., Peden et al., and Pang et al.; Exhibits 2-4) and the consequent loss of the ability to express particular HIV-1 gene products.

Chang's isolate was passaged in the H9/HTLVIII cell line prior to being cloned. (U.S. Appl. 06/643,306 at 6-7; Exhibit 5.) The H9/HTLVIII cell line was established by infection with material from several patients. (Muesing et al. at 452, col. 1.) Muesing et al. indicates that the H9/HTLVIII cell line contains "about five intact provirus copies." (*Id.*) Moreover, Muesing et al. indicates that "these results suggest that two or more distinct virus isolates are integrated stably in the H9/HTLVIII cell line." (*Id.*) Muesing et al. found 68 nucleotide differences between the sequences of proviral and cDNA clones and concluded that "a significant degree of nucleotide heterogeneity is displayed by the proviral and cDNA sequences." (*Id.*) Consequently, multiple nucleotide sequences of HIV-1 were present in the cell line used in the generation of Chang's clones. In light of this evidence, the differences in sequence between applicants' and Chang's clones are likely due to the presence of multiple viruses in the H9/HTLVIII cell line or due to changes in the viruses during passage in the H9/HTLVIII cell line, and not due to "sequencing errors." Accordingly, applicants respectfully request withdrawal of the rejection.

In addition, generic claim 47 is fully supported in the present application and through the priority chain now claimed to Appln. Ser. No. 06/558,109, filed ***December 5,***

1983. The earliest claimed priority date of Chang is August 22, 1984. Consequently, Chang is not effective prior art with respect to claim 47.

Applicants respectfully submit that this application is in condition for allowance. In the event that the Examiner disagrees, he is invited to call the undersigned to discuss any outstanding issues remaining in this application in order to expedite prosecution.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: April 23, 2004

By: _____

Salvatore J. Arrigo
Reg. No. 46,063
Telephone: 202-408-4160
Facsimile: 202-408-4400
E-mail: arrigos@finnegan.com

US006627395B1

(12) **United States Patent**
Montagnier et al.

(10) **Patent No.: US 6,627,395 B1**
(45) **Date of Patent: Sep. 30, 2003**

(54) **METHODS FOR THE PROPAGATION, ISOLATION, IDENTIFICATION, AND PREPARATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)**

(75) Inventors: **Luc Montagnier**, Le Plessis Robinson (FR); **Jean-Claude Chermann**, Elancourt (FR); **Francoise Barre-Sinoussi**, Issy les Moulineaux (FR); **Francoise Brun-Vezinet**, Paris (FR); **Christine Rouzioux**, Paris (FR); **Willy Rozenbaum**, Paris (FR); **Charles Dauguet**, Paris (FR); **Jacqueline Gruest**, L'Hay les Roses (FR); **Marie-Therese Nugeyre**, Paris (FR); **Francoise Rey**, Paris (FR); **Claudine Axler-Blin**, Paris (FR); **Solange Chamaret**, Paris (FR); **Robert C. Gallo**, Bethesda, MD (US); **Mikulas Popovic**, Bethesda, MD (US); **Mangalasseril G. Sarngadharan**, Vienna, VA (US)

(73) Assignees: **Institut Pasteur**, Paris (FR); **The United States of America as represented by the Secretary of the Department of Health and Human Services**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/466,256**

(22) Filed: **Jun. 6, 1995**

Related U.S. Application Data

(63) Continuation of application No. 08/019,297, filed on Feb. 18, 1993, which is a division of application No. 07/876,297, filed on Apr. 30, 1992, now abandoned, which is a continuation of application No. 07/117,937, filed on Nov. 5, 1987, now Pat. No. 5,135,864, which is a continuation of application No. 06/785,638, filed on Oct. 8, 1985, now Pat. No. 4,708,818, which is a continuation of application No. 06/558,109, filed on Dec. 5, 1993, now abandoned.

(30) **Foreign Application Priority Data**

Sep. 15, 1983 (GB) 83 24800
Sep. 15, 1983 (GB) 84/24800

(51) **Int. Cl.**[7] **C12Q 1/70**

(52) **U.S. Cl.** **435/5**; 424/188.1; 424/208.1

(58) **Field of Search** 435/5, 7.1; 424/188.1, 424/208.1

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(List continued on next page.)

Primary Examiner—Laurie Scheiner
Assistant Examiner—J. S. Parkin
(74) *Attorney, Agent, or Firm*—Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.

(57) **ABSTRACT**

The identification, separation, purification, and propagation of the HIV-1 virus is provided. Moreover, the preparation of antigens from HIV-1 is further provided. The identification of HIV-1 involves the purification of a virus sample from lymphocytes and contacting the sample with antibodies, which bind to HIV-1 viruses, is provided. The propagation of HIV-1 virus involves infecting uninfected T lymphocytes with the virus. Moreover, the preparation of antigens from HIV-1 involves the separation of protein components of a purified HIV-1 virus under denaturing conditions.

6 Claims, 1 Drawing Sheet

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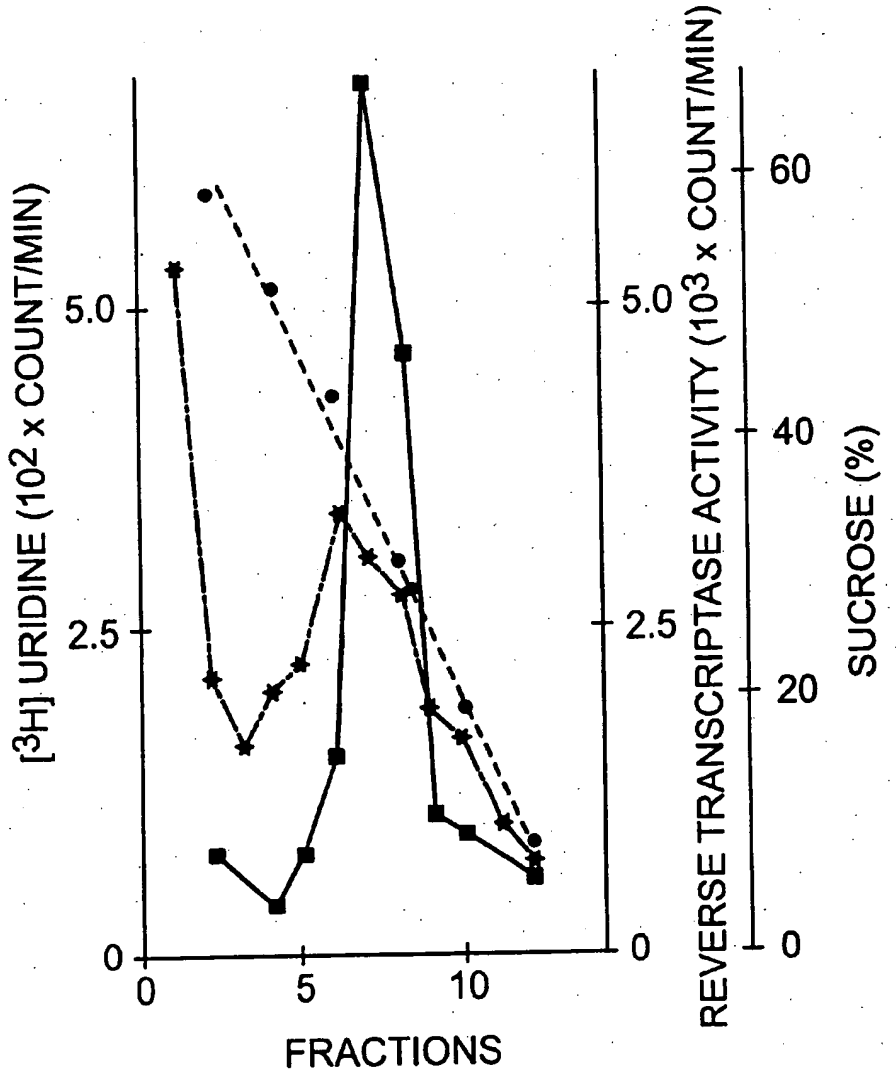


——— MEASURE OF REVERSE TRANSCRIPTASE ACTIVITY ON SUCCESSIVE FRACTIONS OF SUCROSE GRADIENT.

—·—·— MEASURE OF ACID PRECIPITABLE MATERIAL LABELLED WITH [3H] URIDINE.

- - - - - - DENSITY VARIATION OF THE GRADIENT.

FIG. 1

METHODS FOR THE PROPAGATION, ISOLATION, IDENTIFICATION, AND PREPARATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)

This is a continuation of application Ser. No. 08/019,297, filed Feb. 18, 1993, which is a division of application Ser. No. 07/876,297, filed Apr. 30, 1992, now abandoned, which is a continuation application of Ser. No. 07/117,937, filed Nov. 5, 1987, now U.S. Pat. No. 5,135,864, which is a continuation application of Ser. No. 06/785,638, filed Oct. 8, 1985, now U.S. Pat. No. 4,708,818, which is a continuation application of Ser. No. 06/558,109, filed Dec. 5, 1983, now abandoned.

The invention relates to antigens, means and methods for the diagnosis of lymphadenopathy and acquired immune deficiency syndrome.

The acquired immune deficiency syndrome (AIDS) has recently been recognized in several countries. The disease has been reported mainly in homosexual males with multiple partners, and epidemiological studies suggest horizontal transmission by sexual routes as well as by intravenous drug administration, and blood transfusion. The pronounced depression of cellular immunity that occurs in patients with AIDS and the quantitative modifications of subpopulations of their T lymphocytes suggest that T cells or a subset of T cells might be a preferential target for the putative infectious agent. Alternatively, these modifications may result from subsequent infections. The depressed cellular immunity may result in serious opportunistic infections in AIDS patients, many of whom develop Kaposi's sarcoma. However, a picture of persistent multiple lymphadenopathies has also been described in homosexual males and infants who may or may not develop AIDS. The histological aspect of such lymph nodes is that of reactive hyperplasia. Such cases may correspond to an early or a milder form of the disease.

It has been found that one of the major etiological agents of AIDS and of lymphadenopathy syndrom (LAS), which is often considered as a prodromic sign of AIDS, should consist of a T-lymphotropic retrovirus which has been isolated from a lymph node of a homosexual patient with multiple lymphadenopathies. The virus appears to be distinct from the human T-cell leukemia virus (HTLV) family (R. C. Gallo and M. S. Reitz, "J. Natl. Cancer Inst.", 69 (No. 6), 1209 (1982)). The last mentioned virus has been known as belonging to the so-called HTLV-1 subgroup.

The patient was a 33-year-old homosexual male who sought medical consultation in December 1982 for cervical lymphadenopathy and asthenia (patient 1). Examination showed axillary and inguinal lymphadenopathies. Neither fever nor recent loss of weight were noted. The patient had a history of several episodes of gonorrhea and had been treated for syphilis in September 1982. During interviews he indicated that he had had more than 50 sexual partners per year and had travelled to many countries, including North Africa, Greece, and India. His last trip to New York was in 1979.

Laboratory tests indicated positive serology (immunoglobulin G) for cytomegalovirus (CMV) and Epstein-Barr virus. Herpes simplex virus was detected in cells from his throat that were cultured on human and monkey cells. A biopsy of a cervical lymph node was performed. One sample served for histological examination, which revealed follicular hyperplasia without change of the general structure of the lymph node. Immunohistological studies revealed, in paracortical areas, numerous T lymphocytes ($OKT3^+$). Typing of the whole cellular suspension indicated that 62 percent of the cells were T lymphocytes ($OKT3^+$), 44 percent were T-helper cells ($OKT4^+$), and 16 percent were suppressor cells ($OKT8^+$).

Cells of the same biopsed lymph node were put in culture medium with phytohemagglutinin (PHA), T-cell growth factor. (TCGF), and antiserum to human α interferon ("The cells were grown in RPMI-1640 medium supplemented with antibiotics, 10^{-5}M β-mercaptoethanol, 10 percent fetal calf serum, 0.1 percent sheep antibody to human α interferon (neutralizing titer, 7 IU at 10^{-5} dilution and 10 percent TCGF, free of PHA"). The reason for using the antiserum to α-interferon was to neutralize endogenous interferon which is secreted by cells chronically infected by viruses, including retroviruses. In the mouse system, it had previously been shown that anti-serum to interferon could increase retrovirus production by a factor of 10 to 50 (F. Barre-Sinoussi et al., "Ann. Microbiol. (Institut Pasteur)" 130B, 349 (1979). After 3 days, the culture was continued in the same medium without PHA. Samples were regularly taken for reverse transcriptase assay and for examination in the electron microscope.

After 15 days of culture, a reverse transcriptase activity was detected in the culture supernatant by using the ionic conditions described for HTLV-I (B. J. Poiesz et al. "Proc. Natl. Acad. Sci. U.S.A." 77, 7415 (1980)). Virus production continued for 15 days and decreased thereafter, in parallel with the decline of lymphocyte proliferation. Peripheral blood lymphocytes cultured on the same way were consistently negative for reverse transcriptase activity, even after 6 weeks. Cytomegalovirus could be detected, upon prolonged co-cultivation with MRC5 cells, in the original biopsy tissue, but not in the cultured T lymphocytes at any time of the culture.

The invention relates to the newly isolated virus as a source of the above said antigen which will be defined later.

The newly isolated virus, which will hereafter be termed as LAV_1, will however be described first.

The virus is transmissible to cultures of T lymphocytes obtained from healthy donors. Particularly virus transmission was attempted with the use of a culture of T lymphocytes established from an adult healthy donor of the Blood Transfusion Center at the Pasteur Institute. On day 3, half of the culture was co-cultivate with lymphocytes from the biopsy after centrifugation of the mixed cell suspensions. Reverse transcriptase activity could be detected in the supernatant on day 15 of the coculture but was not detectable on days 5 and 10. The reverse transcriptase had the same characteristics as that released by the patient's cells and the amount released remained stable for 15 to 20 days. Cells of the uninfected culture of the donor lymphocytes did not release reverse transcriptase activity during this period or up to 6 weeks when the culture was discontinued.

The cell-free supernatant of the infected co-culture was used to infect 3-day-old cultures of T lymphocytes from two umbilical cords, LC1 and LC5, in the presence of Polybrene (2 μg/ml). After a lag period of 7 days, a relatively high titer of reverse transcriptase activity was detected in the supernatant of both cord lymphocyte cultures. Identical cultures, which had not been infected, remained negative. These two successive infections clearly show that the virus could be propagated on normal lymphocytes from either new-borns or adults.

In the above co-cultures one used either the cells of patient 1 as such (they declined and no longer grew) or cells which had been pre-X-rayed or mitomycin C-treated.

The LAV_1 virus, or LAV_1 virus suspensions, which can be obtained from infected cultures of lymphocytes have

characteristics which distinguish them completly from other HTLV. These characteristics will be referred to hereafter and, when appropriate in relation to the drawing. It shows curves representative of variation of reverse transcriptase activity and [^{3}H] uridine activity respectively versus successive fractions of the LAV_1 virus in the sucrose gradient, after ultra-centrifugation therein of the virus contents of a cell-free supernatant obtained from a culture of infected lymphocytes.

The analysis of LAV_1 virus by resorting to reverse transcriptase activity can be carried out according to the procedure which was used in relation to virus from patient 1, on FIG. 1. The results of the analysis are illustrated on FIG. 1. Cord blood T lymphocytes infected with virus from patient 1 were labelled for 18 hours with [^{3}H] urinde (28 Ci/mmole, Amersham; 20 μCi/ml). Cell-free supernatant was ultra-centrifuged for 1 hour at 50,000 rev/min. The pellet was resuspended in 200 μl of NTE buffer (10 mM tris, pH 7.4, 100 mM NaCl, and 1 mM EDTA) and was centrifuged over a 3-ml linear sucrose gradient (10 to 60 percent) at 55,000 rev/min for 90 minutes in an IEC type SB 498 rotor . Fractions (200 μl) were collected, and 30 μl samples of each fraction were assayed for, DNA fi,A dependant polymerase activity with 5 mM Mg^{2+} and poly(A)-oligo-(dT) as template primer; a 20-μl portion of each fraction was precipitated with 10 percent trichloroacetic acid and then filtered on a 0.45-μm Millipore filter. The ^{3}H labelled acid precipitable material was measured in a Packard β counter.

That the new virus isolate was a retrovirus was further indicated by its density in the above sucrose gradient, which was 1.16, and by its labelling with [^{3}H] uridine (FIG. 1). A fast sedimenting RNA appears to be associated with the LAV_1 virus.

Virus-infected cells from the original biopsy as well as infected lymphocytes from the first and second viral passages were used to determine the optimal requirements for reverse transcriptase activity and the template specificity of the enzyme. The results were the same in all instances. The reverse transcriptase activity displayed a strong affinity for poly(adenylate-oligodeoxy-thymidylate) [poly(A)-oligo $(dT)_{12-18}$], and required Mg^{2+} with an optimal concentration (5 mM) and an optimal pH of 7.8. The reaction was not inhibited by actinomycin D. This character, as well as the preferential specificity for riboseadenylate-deoxythymidylate over deoxyadenylate-deoxythymolylate, distinguish the viral enzyme from DNA-dependent polymerases.

Electron microscopy of ultrathin sections of virus-producing cells shows two types of particles, presumably corresponding to the immature and mature forms of the virus: immature particles are budding at the cell surface, with a dense crescent in close contact with the plasma membrane. Occasionally, some particles remain in this state, while being freed from the cell surface.

Mature particles have a quite different morphology with a small, dense, eccentric core (mean diameter: 41 nM). Most of virions are round (mean diameter: 139 nM) or ovoid, but in some pictures, especially in the particles seen in the original culture from which the virus was isolated, a tailed morphology can also be observed. The latter form can also be observed in cytoplasmic vesicles which were released in the medium. Such particles are also formed by budding from vesicle membranes.

Morphology of mature particles is clearly distinct from HTLV, whose large core has a mean diameter of 92 nM.

Helper T-lymphocytes (Leu 3 cells) form the main target of the virus. In other words the LAV_1 virus has particular tropism for these cells. Leu 3 cells are recognizable by the monoclonal antibodies commercialized by ORTHO under the trademark OKT4. In contrast enriched cultures of Leu 2 cells, which are mainly suppressor or cytotoxic cells and which are recognized by the mono-clonal antibodies commercialized by ORTHO under the trademark OKT8 did not produce, when infected under the same conditions, any detectable RT activity even 6 weeks after virus infection.

In most cases of AIDS, the ratio of $OKT4^+$ over $OKT8^+$ cells which is normally over 1, is depressed to values as low of 0.1 or less.

The LAV_1 virus is also immunologically distinct from previously known HTLV-1 isolates from cultured T lymphocytes of patients with T lymphomas and T leukemias. The antibodies used were specific for the p19 and p24 core proteins of HTLV-1. A monoclonal antibody to p19 (M. Robert-Guroff et al. "J. Exp. Med."154, 1957 (1981)) and a polyclonal goat antibody to p24 (V. S. Kalyanaraman et al. "J. Virol.", 38, 906 (1981)) were used in an indirect fluorescence assay against infected cells from the biopsy of patient 1 and lymphocytes obtained from a healthy donor and infected with the same virus. The LAV_1 virus-producing cells did not react with either type of antibody, whereas two lines of cord lymphocytes chronically infected with HTVL 1 (M. Popovic, P. S. Sarin, M. Robert-Guroff, V. S. Kalyanaraman, D. Mann, J. Minowada, R. C. Gallo, "Science" 219, 856 (1983))and used as controls showed strong surface fluorescence.

In order to determine which viral antigen was recognized by antibodies present in the patient's sera, several immunoprecipitation experiments were carried out. Cord lymphocytes infected with virus from patient 1 and uninfected controls were labelled with [^{35}S] methionine for 20 hours. Cells were lysed with detergents, and a cytoplasmic S10 extract was made. Labelled virus released in the supernatant was banded in a sucrose gradient. Both materials were immunoprecipitated by antiserum to HTVL-1 p24, by serum from patient 1, and by serum samples from healthy donors. Immunocomplexes were analyzed by polyacrylamide gel eletrophoresis under denaturing conditions. A p25 protein present in the virus-infected cells from patient 1 and in LC1 cells infected with this virus, was specifically recognized by serum from patient 1 but not by antiserum to HTLV-1 p24 obtained under similar conditions or serum of normal donors. Conversely the p24 present in control HTLV-infected cell extracts was recognized by antibodies to HTLV but not by serum from patient 1.

The main protein (p25) detected after purification of ^{35}S-methionine-labelled virus has a molecular weight of about 25,000 (or 25KDa). This is the only protein recognized by the serum of patient 1. By analogy with other retroviruses, this major protein was considered to be located in the viral core.

This can be confirmed in immuno-electron microscopy experiments, which show that the patient's serum can agglutinate the viral cores. Conversely, an antiserum raised in rabbit against an ether treated virus did not precipitate the p25 protein.

The viral origin of other proteins seen in polyacrylamide gel electrophoresisof purified virus is more difficult to assess. A p15 protein could be seen after silver staining, but was much weaker after ^{35}S-methionine perhaps due to the paucity of this amino-acid in the protein. In the higher MW range, a contamination of the virus by cellular proteins, either inside or outside the viral envelope, is likely. A 36K and a 42K protein and a 80K protein were constantly formed to be associated with the purified virus and may represent the major envelope proteins.

No. p19 (or having a molecular weight of about 19 mM) was isolated from LAV_1 extracts.

The invention concerns more particularly the extracts of said virus as soon as they can be recognized immunologically by sera of patients afflicted with LAS or AIDS. Needless say any type of immunological assay may be brought into play. By way of example immunofluorescence or immunoenzymatic assays or radio-immunoprecipitation tests are particularly suitable.

As a matter of fact and except under exceptional circumstances, sera of diseased patients do not recognize the intact LAV_1 virus, or viruses having similar phenotypical or immunological properties. The envelope proteins of the virus appeared as not detectable immunologically by the patients' sera. However as soon as the core proteins become exposed to said sera, the immunological detection becomes possible. Therefore the invention concerns all extracts of the virus, whether it be the crudest ones—particularly mere virus lysates —or the more purified ones, particularly extracts enriched in the p25 protein or even the purified p25 protein or in protein immunologically related therewith. Any purification procedure may be resorted to. By way of example only, one may use purification procedures such as disclosed by R. C. Montelaro et al, J. of Virology, June 1982, pp. 1029–1038.

The invention concerns more generally extracts of any virus having similar phenotype and immunologically related to that obtained from LAV_1. Sources of viruses of the LAV type consist of T-lymphocyte cultures isolatable from LAS—and AIDS—patients or, from haemophiliacs.

In that respect other preferred extracts are those obtained from two retroviruses obtained by propagation on normal lymphocytes of the retroviruses isolated from:

1) lymph node lymphocytes of a caucasian homosexual with multiple partners, having extensive Kaposi sarcoma lesions and severe lymphopenia with practically no $OKT4^+$ lymphocytes in his blood;
2) blood lymphocytes of a young B haemophiliac presenting neurotoxoplasmosis and $OKT4^+/OKT8^+$ ratio of 0.1.

These two retroviruses have been named IDAV1 and IDAV2 respectively (for Immune Deficiency Associated Virus). Results of partial characterization obtained so far indicate similarity—if not identity—of IDAV1 and IDAV2 to LAV1:

- same ionic requirements and template specificities of reverse transcriptase,
- same morphology in ultrathin sections,
- antigenically related p25 proteins: serum of LAV_1 patient immunoprecipitates p25 from IDAV1 and IDAV2; conversely, serum from IDAV2 patient immunoprecipitates LAV_1 p25.

IDAV1 patient serum seemed to have a lower antibodies titer and gave a weak precipitation band for LAV1 and IDAV1 p25 proteins. The p25 protein of IDAV1 and IDAV2 was not recognized by HTLV p24 antiserum.

These similarities suggest that all these three isolates belong to the same group of viruses.

The invention further relates to a method of in vitro diagnosis of LAS or AIDS, which comprises contacting a serum or other biological medium from a patient to be diagnosed with a virus extract as above defined and detecting the immunological reaction.

Preferred methods bring into play immunoenzymatic or immunofluorescent assays, particularly according to the ELISA technique. Assays may be either direct or indirect immunoenzymatic or immunofluorescent assays.

Thus the invention also relates to labelled virus extracts whatever the type of labelling: enzymatic, fluorescent, radioactive, etc.

Such assays include for instance:

- depositing determined amounts of the extract according to the invention in the wells of titration microplate;
- introducing in said wells increasing dilutions of the serum to be diagnosed;
- incubating the microplate;
- washing the microplate extensively;
- introducing in the wells of the microplate labelled antibodies directed against blood immunoglobulines the labelling being by an enzyme selected among those which are capable of hydrolysing a substrate, whereby the latter then undergoes a modification of its absorption of radiations, at least in a determined wavelength band and
- detecting, preferably in a comparative manner with respect to a control, the amount of substrate hydrolysis as a measure of the potential risks or effective presence of the disease.

The invention also relates to kits for the above said diagnosis which comprise:

- an extract or more purified fraction of the abovesaid types of viruses, said extract or fraction being labelled, such as by a radioactive, enzymatic or immunofluorescent label;
- human anti-immunoglobulins or protein A (advantageously fixed on a water-insoluble support such as agarose beads);
- a lymphocyte extract obtained from a healty person;
- buffers and, if appropriate, substrates for the vizualization of the label.

Other features of the invention will further appear as the description proceeds of preferred isolation and culturing procedures of the relevant virus, of preferred extraction methods of an extract suitable as diagnostic means, of a preferred diagnosis technique and of the results that can be achieved.

1. VIRUS PROPAGATION

Cultured T-lymphocytes from either ombilical cord or blood, or also bone marrow cells from healthy, virus negative, adult donors are suitable for virus propagation.

There is however some variation from individual to individual in the capacity of lymphocytes to grow the virus. Therefore, it is preferable to select an adult healthy donor, which had no antibodies against the virus and whose lymphocytes repeatly did not release spontaneously virus, as detected by reverse transcriptase activity (RT) nor expressed viral proteins.

Lymphocytes of the donor were obtained and separated by cytophoresis and stored frozen at −180° C. in liquid nitrogen, in RPMI 1640 medium, supplemented with 50% decomplemented human serum and 10% DMSO until used.

For viral infection, lymphocytes were put in culture (RPMI 1640 medium) with phytohaemagglutinin (PHA) at the concentration of 5.10^6 cells/ml for 3 days.

Then, the medium was removed and cells resuspended in viral suspension (crude supernatant of virus-producing lymphocytes, stored at −80° C.). Optimal conditions of cell/virus concentrations were 2.10^6 cells for 5 to 10,000 cpm of RT activity, the latter determined as previously described. After 24 hours, cells were centrifuged to remove

the unadsorbed virus and resuspended in culture PHA-free medium and supplemented with PHA-free TCGF (Interleukin 2): (0.5–1 U/ml, final concentration), POLYBREN (Sigma) 2 μg/ml and anti-interferon α sheep serum, inactivated at 56° C. for 30 minutes (0.1% of a serum which is able to neutralize 7 U of α leucocyte interferon at a 1/100,000 dilution).

Virus production was tested every 3 days by RT activity determination on 1 ml samples.

The presence of anti-interferon serum is important in virus production: when lymphocytes were infected in the absence of anti-human-α-interferon serum, virus production, as assayed by RT activity, was very low or delayed. Since the sheep antiserum used was raised against partly purified a leucocyte interferon, made according to the Cantell technique, the role of components other than interferon cannot be excluded.

Virus production starts usually from day 9 to 15 after infection, and lasts for 10–15 days. In no cases, the emergence of a continuous permanent line was observed.

2. VIRUS PURIFICATION

For its use in ELISA, the virus was concentrated by 10% Polyethylenglycol (PEG 6000) precipitation and banded twice to equilibrium in a 20–60% sucrose gradient. The viral band at density 1.16 is then recovered and usable as such for ELISA assays.

For use in RIPA radio-immune precipitation assay, purification in isotonic gradients of Metrizamide (sold under the trademark NYCODENZ by Nyegaard, Oslo) were found to be preferable. Viral density in such gradients was very low (1.10–1.11).

Metabolic labelling with ^{35}S-methionine of cells and virus (RIPA) followed by polyacrylamide gel electrophioresis were performed as above described except the following modifications for RIPA: virus purified in NYCODENZ was lysed in 4 volumes of RIPA containing 500 U/ml of aprotinin. Incubation with 5 μl or serum to be tested was made for 1 hour at 37° C. and then 18 hours at +4° C. Further incubation of the immunocomplexes with protein A SEPHAROSE beads was for 3 hours at +4° C.

3. PREPARATION OF THE VIRUS EXTRACT FOR ELISA ASSAYS

Virus purified in sucrose gradient as above described, is lysed in RIPA buffet (0.5% SDS) and coated on wells of microtest plates (Nunc).

Preferred conditions for the ELISA assay are summed up hereafter.

After addition to duplicate wells of serial dilutions of each serum to be tested, the specifically fixed IgGs are revealed by goat anti-human IgG coupled with peroxydase. The enzymatic reaction is carried out on ortho-phenylenediamine as substrate and read with an automatic spectrophotometer at 492 nM.

On the same plate each serum is tested on a control antigen (a crude cytoplasmic lysate of uninfected T-lymphocytes from the same donor) is used in order to eliminate unspecific binding, which can be high with some sera.

Sera are considered as positive (antibodies against the virus) when the difference between O.D. against the viral antigen and O.D. against control cellular antigen was at least 0.30.

Hereafter there is disclosed a specific test for assaying the above mentionned disease or exposure to disease risks.

Method

This ELISA test is for detecting and titration of seric anti-retrovirus type LAV antibodies.

It comprises carrying out a competition test between a viral antigen (cultivated on T lymphocytes) and a control antigen constituted by a lysate of the same though non-infected lymphocytes.

The binding of the antibodies on the two antigens is revealed by the use of a human antiglobulin labelled with an enzyme which itself is revealed by the addition of a corresponding substrate.

Preparation of the Viral Antigen

The cellular cultures which are used are T lymphocytes of human origin which come from:

umbilical cord blood,

bone marrow,

blood of a healthy donnor.

After infection of the cells by the virus, the supernatant of the infected cell culture is used. It is concentrated by precipitating with 10% PEG, then purified (two or three times) on a (20–60%) sucrose gradient by ultracentrifugation to equilibrium.

The viral fractions are gathered and concentrated by centrifugation at 50,000 rotations per minute for 60 minutes.

The sedimented virus is taken in a minimum volume of buffer NTE at pH 7.4 (Tris 0.01 M, NaCl 0.1 M, EDTA 0.001 M).

The proteic concentration is determined by the Lowry method.

The virus is then lysed by a (RIPA+SDS) buffer (0.5% final) for 15 minutes at 37° C.

Preparation of the Control Antigen

The non-infected lymphocytes are cultured according to the preceeding conditions for from 5 to 10 days. They are centrifuged at low speed and lysed in the RIPA buffer in the presence of 5% of the product commercialized under the name of ZYMOFREHN (Spécia) (500 μ/ml). After a stay of 15 minutes at 4° C. with frequent stirrings with vortex, the lysate is centrifuged at 10,000 rotations per minute. The supernatant constitutes the control antigen. Its concentration in protein is measured by the Lowry method.

Reagents

1 —Plates=NUNC—special controlled ELISA

2 —Buffer PBS: pH 7.5

3 —TWEEN 20

4 —Carbonate buffer: pH=9.6 (CO_3Na_2=0.2 M (CO_3HN_a=0.2 M

5 —Non foetal calf serum: which is stored in frozen state (BIOPRO)

6 —Bovine serum albumine (BSA) SIGMA (fraction V)

7 —Human anti IgG (H+L) labelled with peroxydase PASTEUR, in tubes of 1 ml preserved at 4° C.

8 —Washing buffer=PBS buffer, pH 7.5+0.05% TWEEN 20

Dilution of the conjuguate is carried out at the dilution indicated in PBS buffer+TWEEN 20 (0.05%)+bovine albumine 0.5 g per 100 ml

9—Dilution buffer of sera=PBS buffer+0.05%TWEEN 20 +0.5 g BSA bovine serum albumine per 100 ml

10—Substrat=OPD

Citrate buffer pH=5.6 trisodic citrate ($C_6H_5Na_4O_3$, $2H_2O$), 0.05 M; citric acid ($C_6H_8O_7$, $1H_2O$), 0.05 M.

Hydrogen peroxyde=at 30% (110 volumes)—used at 0.03% when using citrate buffer.

Orthophenylene diamine=SIGMA

75 mg per 25 ml of buffer—which is diluted in buffer extemporaneously.

Preparation of Tie Plates

The plates which are used have 96 U-shaped wells (NUNC=ELISA). They include 12 rows of 8 wells each, numbered from 1 to 12.

The distribution of antigens is as follows:

100 μl of the viral antigen, diluted in carbonate buffer at pH 9.6, are deposited in each of the wells of rows (marked θ)

1-2-5-6-9-10

100 μl of the control antigen, diluted in carbonate buffer at pH 9.6, are deposited in each of the wells of rows (marked θ)

3-4-7-8-11-12.

The dilution of the viral antigen is titrated at each viral production. Several dilutions of viral antigen are tested and compared to positive and negative known controls (at several dilutions) arid to human anti-IgG labelled with peroxydase, the latter being also tested at several dilutions.

As a rule, the proteic concentration of the preparation is of 5 to 2.5 μg/ml.

The same proteic concentration is used for the control antigen.

The plates are closed with a plastic lid and are incubated overnight at 4° C.

Then they are put once in distilled water and centrifiuged. The wells are then filled with 300 μl of non foetal calf serum at 20% in PBS buffer.

The incubation lasts 2 hours at 37° C. (covered plates).

The plates are washed 3 times in PBS buffer with TWEEN 20, 0.05% (PBS-tw buffer)

first washing 300 μl

second and third washing 200 μl/well.

The plates are carefully dried and sealed with an adhesive plastic film. They can be stored at −80° C.

ELISA Reaction: Antibody Titer Assay

After defreezing, the plates are washed 3 times in PBS-TWEEN. They are carefully dried.

The positive and negative control sera as well as the tested sera are diluted first in the tube, with PBS-TWEEN containing 0.5% bovine albumine.

The chosen dilution is 1/40.

100 μl of each serum are deposited in duplicate on the viral antigen and in duplicate on the control antigen.

The same is carried out for the positive and negative diluted sera.

100 μl of PBS+TWEEN+bovine serum albumine are introduced in two wells θ and in two wells θ to form the conjugated controls.

The plates equiped with their lids are incubated for 1 h 30 at 37° C.

They are washed 4 times in PBS+TWEEN 0.05%.

100 μl of human anti-IgG (labelled with peroxydase) at the chosen dilution are deposited in each well and incubated at 37° C.

The plates are again washed 5 times with the (PBS+TWEEN) buffer. They are carefully dried.

Revealing the enzymatic reaction is carried out by means of a orthophenylene-diamine substrate (0.05% in citrate buffer pH 5.6 containing 0.03% of H_2O_2).

100 μl of substrate are distributed in each well.

The plates are left in a dark room 20 minutes at the laboratory temperature.

Reading is carried out on a spectrophotometer (for microplates) at 492 nm.

Sera deemed as containing antibodies again the virus are those which give a ODD (optical density difference=optical density of viral antigen less optical density of control antigen) equal or higher to 0.30.

This technique enables a qualitative titration as well as a quantitative one. For this purpose, it is possible either to use several dilutions of the serum to be assayed, or to compare a dilution of the serum with a range of controls tested under the same conditions.

The table hereafter provides first results of serological investigations for LAV antibodies, carried out by using the above exemplified ELISA assay.

FIRST RESULTS OF SEROLOGICAL INVESTIGATIONS FOR LAV ANTIBODIES IN FRANCE

	Total	ELISA-LAV		ELISA-HTLV1* * (Biotech)	
	examined	positive	% positive	positive	% positive
Lympha-denopathy patients*	35	22	(63)	5* * *	(14)
Healthy homosexuals	40	7	(17)	1	(3)
Control population	54	1	(1.9)	0	(<2.6)

*28 homosexuals
3 Haitians (1 woman)
4 toxicomans (2 women)

* *The number of positive sera is probably overestimated in this test, since no control of unspecific binding could be done.

* * *Out of the 5 LAS HTLV1 positive, 3 were born in Haiti, 1 had stayed for a long time in Haiti and 1 had made several travels to USA. All of them had also antibodies against LAV.

The table shows clearly high prevalence of LAV antibodies in the homosexual patients with LAS, the very low incidence in the normal population and also a moderate spread of virus infection in still healthy homosexuals. In the latter group, all the positive individuals had a high number of partners (>50 per year). The incidence of HTLV antibodies was very low in all three groups (determined by using a commercial ELISA test (Biotech)). The groups of AIDS patients gave less interpretable results: approximatively 20% had LAV antibodies, but some of the sera were taken at a very late stage of the disease, with a possible negativation of the humoral response.

It should further be mentionned that lymphocytes of all LAS patients do not produce detectable amounts of LAV-type virus. Particularly cells of lymph nodes from 6 more LAS patients were put in culture and tested for virus production as described for patient 1. No virus release could be detected by RT activity. However, a p25 protein recognized by the serum of the first patient could be detected in cytoplasmic extracts of the T-cells labelled with ^{35}S-methionine in 3 other cases. This suggests partial expression of a similar virus in such cases. Moreover, all (6/6) of these patients had antibodies against LAV p25 proteins, indicating that they all had been infected with a similar or identical virus.

Interestingly, in lymphocytes of one of the patients (patient 2), there was a weak but definite immunoprecipitation of a band of similar size (p24–25) with goat antiserum

raised against HTLV 1. Similarly, the patient's serum had antibodies against both HTLV and LAV, suggesting a double infection by either viruses. Such cases seem rattler un frequent.

The invention finally also relates to the biological reagents that can be formed by the LAV extracts containing the p25 protein or by the purified p25 protein, particularly for the production of antibodies directed against p25 in animals or of monoclonal antibodies. These antibodies are liable of forming useful tools in the further study of antigenic determinants of LAV viruses or LAV-related viruses.

It is acknowledged that the OKT designations which have been used with respect to the designation of some sub-sets of lymphocytes or related monoclonal antibodies by way ease of language, should in no way be opposed to the validity of any corresponding trademark, whether registered or not by its owner.

It should further be mentionned that the viral extracts, particularly viral lysates or enriched fractions can also be defined by reference to their immunological relationship or similitude with the extracts or enriched fractions containing a p25 protein as obtainable from the strain LAV1, IDAV1 or IDAV2. Thus any protein fraction which is capable of giving similar patterns of immunological reaction as do the protein extracts of LAV1, IDAV1 or IDAV2 with the same sera, must be considered as equivalent therefor and, accordingly, be deemed as encompassed by the scope of the claims which follow. A similar conclusion extends of course to the diagnostic means (process and kits) which may make use of such equivalent protein extracts.

The LAV1 virus has been deposited at the "Collection Rationale des Cultures de Micro-organismes" (C.N.C.M.) under no. I-232 on Jul. 15, 1983 and IDAV1 and IDAV 2 viruses have been deposited at the C.N.C.M. on Sep. 15, 1983 under no. I-240 and I-241, respectively. The invention encompasses as well the extracts of mutants or variants of the above deposited strains as long as they possess substantially the same immunological properties.

We claim:

1. A method for preparing and detecting HIV-1 RNA from a lysate of an HIV-1 virus, said method comprising:

(a) providing a biological sample that comprises human CD4+ lymphocytes infected with HIV-1 virus;

(b) separating said virus from said human CD4+ lymphocytes;

(c) centrifuging said separated virus to form a fraction comprising concentrated virus;

(d) isolating said fraction comprising concentrated virus;

(e) lysing said virus;

(f) precipitating the RNA of said virus; and

(g) detecting said viral RNA.

2. The method of claim 1, wherein said method comprises banding said virus on a sucrose gradient or a metrizamide gradient.

3. The method of claim 1, wherein said method comprises pelleting said virus.

4. The method of claim 3, wherein said method comprises precipitating said virus with polyethylene glycol.

5. The method of claim 1, wherein the virus is lysed with SDS.

6. The method of claim 1, wherein said nucleic acid is precipitated with trichloroacetic acid.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

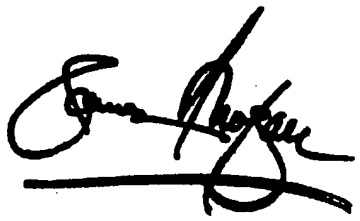
PATENT NO. : 6,627,395 B1
DATED : September 30, 2003
INVENTOR(S) : Luc Montagnier et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item [30], **Foreign Application Priority Data**, delete the second occurrence of "Sep. 15, 1983 (GB) . . . 84/24800".

Signed and Sealed this

Thirtieth Day of December, 2003



JAMES E. ROGAN
Director of the United States Patent and Trademark Office

51481

PATENT OR DESIGN: SOLE OR JOINT

ASSIGNMENT

FOR UNFILED APPLICATION FOR UNITED STATES PATENT

(Sole or Joint Inventors)

FULL NAME(S) AND POST OFFICE ADDRESS(S) OF INVENTOR(S) including country)

WHEREAS:

- ALIZON Marc, 71, rue du Cardinal Lemoine 75005 PARIS (France)
- SONIGO Pierre 23, rue Gutemberg 75015 PARIS (France)
- STEWART Cole
  4Bis Villa Denise 92320 CHATILLON (France)
- DANOS Oliver 1, Place Rollet 75015 PARIS (France)
- WAIN-HOBSON Simon 3, rue Jean de la Fontaine
  78180 MONTIGNY LES BRETONNEUX (France)

(hereinafter referred to as ASSIGNOR), have invented and own a certain invention entitled:

TITLE OF INVENTION

CLONED DNA SEQUENCES RELATED TO THE GENOMIC RNA OF LYMPHADENOPATHY-ASSOCIATED VIRUS(LAV) AND... RNA

for which application for Letters Patent of the United States has been executed on ~~even date herewith~~, August 30, 1985,

FULL NAME AND ADDRESS (including country) OF ASSIGNEE

WHEREAS: INSTITUT PASTEUR
25-28, rue du Dr. Roux
75724 PARIS CEDEX 15 (France) and

CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
15, Quai Anatole France
75007 PARIS (France)

(hereinafter referred to as ASSIGNEE), is desirous of acquiring the entire interest in, to and under said invention and the United States Letters Patent to be obtained therefor;

NOW, THEREFORE, TO ALL WHOM IT MAY CONCERN: Be it known that in consideration of the payment by ASSIGNEE to ASSIGNOR of the sum of One Dollar ($1.00), the receipt of which is hereby acknowledged, and for other good and valuable consideration, ASSIGNOR hereby sells, assigns and transfers to ASSIGNEE the full and exclusive right, title and interest to said invention and all Letters Patent of the United States to be obtained therefor on said application or any continuation, division, renewal, substitute or reissue thereof for the full term or terms for which the same may be granted.

ASSIGNOR hereby covenants that no assignment, sale, agreement or encumbrance has been or will be made or entered into which would conflict with this assignment and sale;

ASSIGNOR further covenants that ASSIGNEE will, upon its request, be provided promptly with all pertinent facts and documents relating to said application, said invention and said Letters Patent as may be known and accessible to ASSIGNOR and will testify as to the same in any interference or litigation related thereto and will promptly execute and deliver to ASSIGNEE or its legal representative any and all papers, instruments or affidavits required to apply for, obtain, maintain and enforce said application, said invention and said Letters Patent which may be necessary or desirable to carry out the purposes hereof.

DATE OF SIGNING: (It must be the same or the date of signing this declaration and one of the patent or sign application.)

IN WITNESS WHEREOF, I/We have hereunto set hand and seal this 7-2-1986
(Date of Signing)

SIGNATURE(S) of signature(s) must correspond with the name(s) of the inventor(s) above.

Pierre SONIGO (Signature)

(Signature)

Marc Alizon

(Signature)

Simon Wain-Hobson

NOTE: No witnessing, notarization or legalization is necessary, but can be included if desired as extra